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Bioprocess engineering of microalgae to produce a variety of consumer products

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ABSTRACT

Microalgae biotechnology has recently emerged into the lime light owing to numerous consumer products that can be harnessed from microalgae. Product portfolio stretches from straightforward biomass production for food and animal feed to valuable products extracted from microalgal biomass, including triglycerides which can be converted into biodiesel. For most of these applications, the production process is moderately economically viable and the market is developing. Considering the enormous biodiversity of microalgae and recent developments in genetic and metabolic engineering, this group of organisms represents one of the most promising sources for new products and applications. With the development of detailed culture and screening techniques, microalgal biotechnology can meet the high demands of food, energy and pharmaceutical industries. This review article discusses the technology and production platforms for development and creation of different valuable consumer products from microalgal biomass.

Contents

1. Introduction

Microalgae consist of a wide range of autotrophic organisms which grow through photosynthesis just like land based plants. Their unicellular structure allows them to easily convert solar energy into chemical energy. A growing range of studies have been conducted to explore the techniques, procedures and processes of

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Table 1
A selection of microalgae species and their method of cultivation.

Species/group	Method of cultivation	References
Spirulina, *Arthrospira platensis*/Cyanophyta	Open ponds, natural lakes	[8]
Chlorella vulgaris/Chlorophyta	Open ponds, basins, glass-tube, PBR	[9,10]
Dunaliella salina/Chlorophyta	Open ponds, PBR	[11,12]
Haematococcus pluvialis/Chlorophyta	Open ponds, PBR	[13,14]
Porphyridium cruentum/Rhodophyta	Tubular PBR	[15,16]
Muriellopsis sp./Chlorophyta	Open ponds, PBR	[17,18]

Modified from Pulz and Gross [7].

producing large quantities of microalgae biomass [1,2]. There are two most commonly used techniques to cultivate microalgae. These are open raceway pond system and closed photobioreactor system. The open pond system is less favourable due to limitation in controlling contaminations from predators whilst the photobioreactors provide an easy system of controlling nutrients for growth, cultivation parameters such as temperature, dissolved CO_2 and pH, and to prevent contaminations [3]. However, photobioreactors have a high initial cost and are very specific to the physiology of microalgae strain being cultivated. Therefore, microalgae production facility is an important factor to be considered for the optimum production of a specific microalgal species (spp). Table 1 shows the cultivation system for various microalgal spp. Methods such as flocculation, centrifugation and filtration are used unaided to dewater the algae biomass [4–6]. An optimum dewatering technique should be applicable to a wide range of microalgal strains, have high biomass recovery and also cost effective. It is therefore important to understand various technologies in cultivating and dewatering microalgae in order to maximise the production of microalgae at low cost.

Microalgae biotechnology has been developed for different commercial applications. As photosynthetic organisms, microalgae contain chlorophyll that can be used for food and cosmetic purposes [2]. They can also be used in pharmaceutical industries, as some species of microalgae produce bioactive compounds such as antioxidants, antibiotics and toxins [19]. Besides, microalgae are used as nutrient supplements for human consumption due to high in protein, vitamins and polysaccharides contents [20]. Some microalgae species contains high levels of lipids which can be extracted and converted into biofuels. The common methods that have been employed to extract the lipids from microalgae include oil press, solvent extraction, supercritical fluid extraction and ultrasound [21–23]. The extracted lipids can further be trans-esterified into biodiesel [22]. Furthermore, the waste biomass that is left behind after the lipids have been extracted could be converted to produce different types of biofuels such as biomethane [24]; bioethanol [25]; and biohydrogen [26].

Microalgae have displayed the potential to curb emerging environmental problems, such as the greenhouse effect and industrial water pollution. Microalgae can fix carbon dioxide released from power plants by photosynthesis and produce nutrients efficiency at a minimal cost [27]. In addition, some species of microalgae have the ability to fix nitrogen and absorb heavy metals and phosphorus [28,29]. The above-mentioned scenarios show that microalgae can provide possible solutions to some of environmental problems as well as creating valuable consumer products. Fig. 1 shows a conceptual schematic diagram for the development of products from microalgae through carbon recycling.

This article discusses the different cultivation, dewatering and processing methods of microalgae in order to produce a variety of consumer products. These commodities include renewable fuels, fine organic chemicals, as well as food supplements for human consumption. The potential of microalgae in resolving the environmental problems is also addressed in this article. However, the economics, sustainability and environmental perspectives in producing each of the commodities are outside the scope of this study.

2. Cultivation of microalgae

2.1. Open ponds

The use of open ponds as a method for the cultivation of microalgae is quite common. Open ponds come in many different shapes and forms, each having certain advantages and drawbacks. The types of ponds that are currently used in research and industry include raceway ponds, shallow big ponds, circular ponds tanks and closed ponds. The location in which the pond is situated is a critical factor in determining the type of pond selected, algal strain

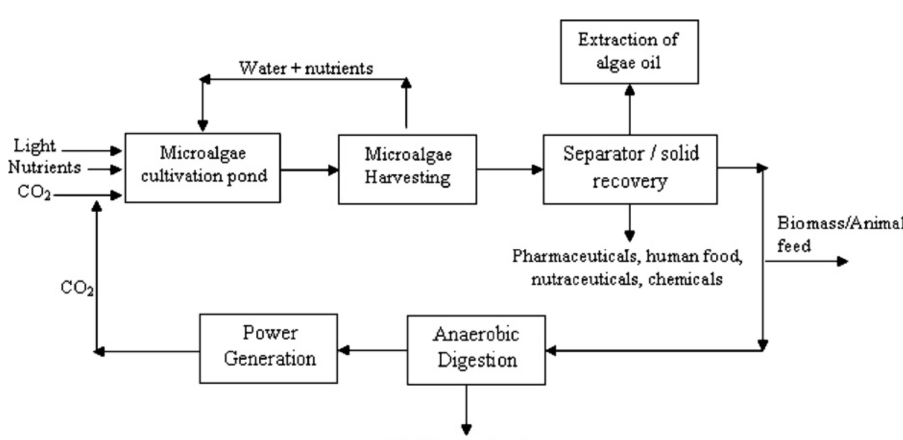


Fig. 1. Schematic diagram for microalgae biotechnology (modified from Chisti [27]).

and amount of light for photosynthesis. Due to the lack of control involved with open systems, the pond becomes a function of the local climate, thus the location significantly contributes to the success of the cultivation [30]. Open ponds are limited by key growth parameters including light intensity, temperature, pH and dissolved oxygen concentration. Contamination by predators is another issue involved with open ponds. Contamination can limit the cultivation system to algal strains which can only grow under severe conditions.

Several studies have assessed the possibility of microalgae cultivation using the open ponds system. Lee [31] reported that only a limited range of microalgae species resistant to be cultured in open ponds due to severe culture environment (*Dunaliella*-high salinity, *Spirulina*-high alkalinity, and *Chlorella*-high nutrition). *Dunaliella salina* is one of the most successful species that have been used for production of carotenoids which protect *D. salina* against the intense of climate condition in open ponds system [11]. Hase et al. [32] achieved a stable photosynthetic efficiency of *Chlorella* sp. and *Chlorophyta* sp. in raceway system. Moreover, Blanco et al. [17] used *Muriellopsis* sp. to produce lutein rich cells in the open tank agitated with a paddlewheel. These cells can be used as food dyes, and as feed additives in aquaculture and poultry farming. They found that the yield of cells was quite similar with closed system.

Furthermore, cost of a cultivation system of microalgae is a vital factor when comparing open and closed cultivation systems. It is well known that the cost involved with cultivation ponds are significantly less than that of closed systems. The construction, operating and maintenance costs of cultivation ponds are lower than photobioreactor options (www.oilgae.com), they are less technical in design and are more scalable. Although the ponds contain a relatively lower biomass concentration, the previously mentioned features of the pond make it a competitive cultivation option.

2.2. Photobioreactors

Nowadays, the research on designing of photobioreactors to cultivate photosynthetic cells of microalgae is extensively investigated. Photobioreactor gives a better control on most of parameters compared to open pond systems [27]. Table 2 represents comparison of microalgae cultivation techniques; open ponds and photobioreactors. The controlled environment of photobioreactor allows a higher productivity to be achieved. Productivity is the most important indicator for success of technology behind a bioreactor. It is difficult to compare productivity of bioreactors because of different strains and scales of microalgae being used. Basically, photobioreactor comes in a different range of designs: tubular and plate-types. In comparison to other photobioreactors, tubular reactors are considered to be more appropriate for outdoor cultivation. The large illumination surface of the reactor which is created by translucent tubing is the main factor behind its outdoor suitability. The tubing can be arranged in various configurations and the appropriateness of the configuration depends on the specifications of the system. Common configurations include straight line and coiled tubing [3]. The geometery of the reactor is also important, as tubular reactors can be configured in a vertical, horiztional or inclined plane. The major difference between the configurations is that the vertical design allows greater mass transfer and a decrease in energy usage, while the horizontal reactor is more scaleable, but requires a large area of land [3].

A number of studies have been reported the application of tubular photobioreactors in culturing microalgae: vertical [34], horizontal [35] and helical [36]. On the other hand, flat-plate photobioreactor is broadly in use due to narrow light path, which helps maintaining higher cell densities by more than an order of magnitude compared to other photobioreactors [37]. Additionally, this type of reactors are favourable due to (1) low power energy consumption and high mass transfer capacity, (2) reduction in oxygen build up, (3) no dark volumes compared in large degassers and other photobioreactors and (4) high photosynthetic efficiency [38]. The appropriate reactor design is required to obtain the maximal cell mass. Various designs of flat-plate photobioreactors to cultivate microalgae have been constructed: glass types [39], thick transparent PVC materials [10], V-shaped [40] and inclined [37]. The glass and PVC types are more transparent for maximum light penetration while other designs are cheap and easy to construct. Fig. 2 represents schematic diagrams of tubular photobiorectors and raceway pond type.

3. Dewatering of microalgal cultures

3.1. Flocculation

Flocculation is used to amass microalgae cells from the broth. Flocculation can be used as an initial dewatering step that will significantly enhance the ease of further processing. Microalgae carry a negative charge which prevents them from self-aggregation within suspension. The surface charge on the algae can be countered by the addition of chemicals known as flocculants. These cationic chemicals coagulate the algae without affecting the composition and toxicity of the product. Types of flocculants include $Al_2(SO_4)_3$ (aluminium sulphate), $FeCl_3$ (ferric chloride) and

Table 2
Comparison chart of algae cultivation method.

Factor	Open ponds	Photobioreactors
Space required	High	Low
Water loss	Very high	Low
CO_2-loss	High, depending on pond depth	Low
Oxygen concentration	Low due to continuous spontaneous outgassing	Build-up occurred requires gas exchange device
Temperature	Highly variable	Required cooling
Shear	Low	High
Cleaning	None	Required due to wall growth and dirt
Contamination	High	None
Evaporation	High	No evaporation
Biomass quality	Variable	Reproducible
Harvesting cost	High	Lower
Microbiology safety	None	UV
Automatic cooling system	None	Built in
Automatic heating system	None	Built in
Air pump	Built in	Built in
Energy requirement (W)	4000	1800

Modified from Pulz [33].

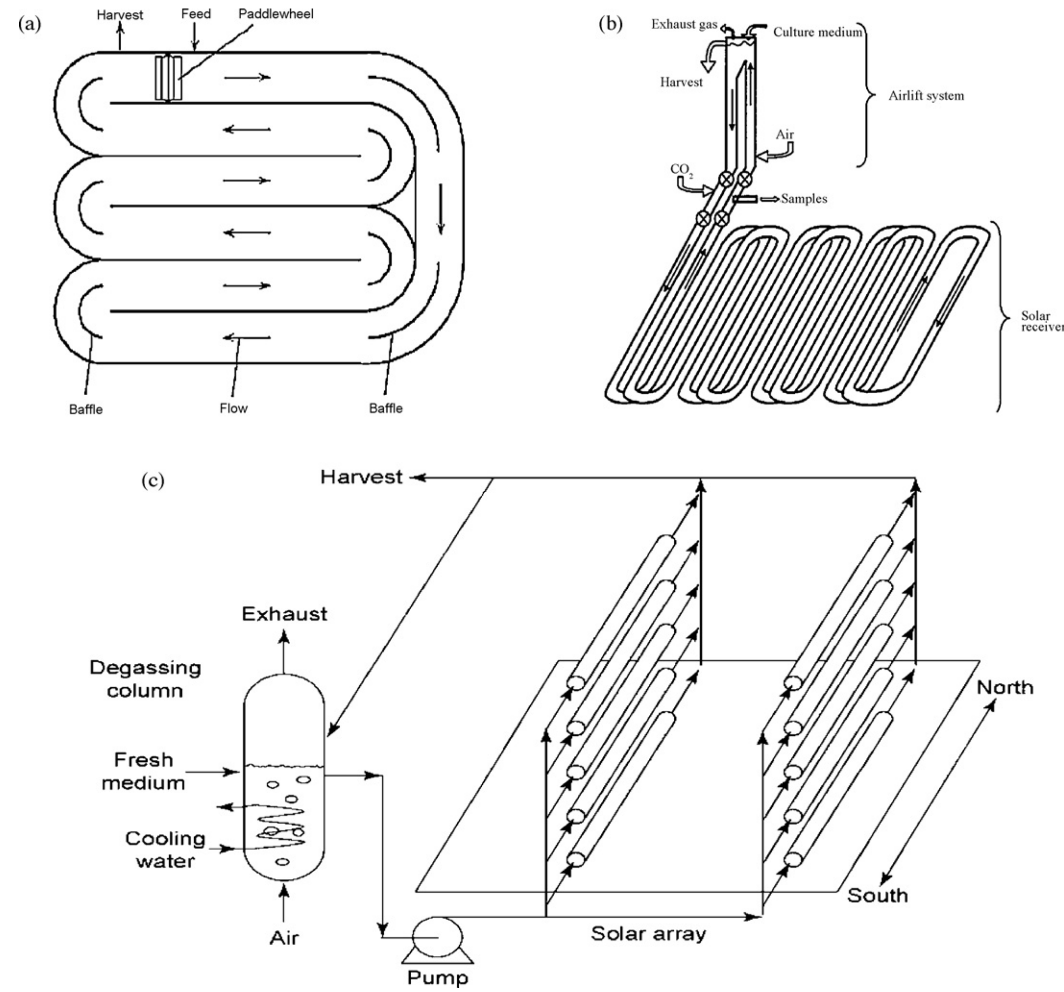


Fig. 2. Reactor configurations for microalgae cultivation (a) raceway pond [41]; (b) external loop tubular reactor [42]; (c) horizontal tubular reactor [41].

Fe_2 $(SO4)_3$ (ferric sulphate). These multivalent salts are commonly used and vary in effectiveness, which is directly related to the ionic charge of the flocculant. Knuckey et al. [5] used Fe^{3+} flocs with induced pH to harvest various kinds of algae and achieved the efficiencies around 80%. Chen et al. [43] reported that flocculation efficiency by ferric chloride using *Aphanothece halophytica* was suppressed by amount of polysaccharide released. Extra amounts of ferric chloride are needed in order to maintain flocculation efficiency. Also, Molina Grima et al. [42] reviewed usage of metal salts as effective flocculants to separate algae. Aluminium sulphate ($Al_2(SO_4)_3$, alum) and ferric sulphate ($Fe_2(SO_4)_3$) are also used as the flocculants agents.

The other type of flocculants used are polyelectrolytes, which are cationic polymers. Polymer flocculants have the advantage of phyisically linking cells together. The extent of aggregation by the polyelectrolytes will depend on the specific properties of the polymer. Key polymer chacteristics include charge, molecular weight and concentration. Increasing the molecular weight and charge on the polymers has been shown to increase their binding capabitites. The type of polymer chosen will also depend on the properties of the algal culture, such as charge in broth, pH and biomass concentration. Tenney et al. [44] reported cationic polyelectrolyte gave better flocculation results for *Chlorella*, whereas no flocculation was found with the anionic polyelectrolyte. The organic flocculants are reported to give an advantage in terms of less sensitivity of pH, wide range of applications and require lower dosage for flocculation process [45]. Furthermore, a commercial product called "chitosan", commonly used for water purification, can also be used as flocculent. However, it is too expensive to be used for economic algae dewatering. Further, brackish or saline water requires an additional chemical flocculant to induce flocculation. Divakaran and Pillai [46] studied flocculation of *Spirulina*, *Oscillatoria*, *Chlorella* and *Synechocystis* using chitosan. The proportional correlation between rate of flocculation and concentrations of chitosan were observed. Higher concentration of chitosan resulted in faster settling rate of algae. However, variable amounts of chitosan will be required for different algal species, thus resulting in varied flocculation efficiencies. Heasman et al. [6] tested chitosan as flocculant for *Tetraselmis chui*, *Thalassiosira pseudonana* and *Isochrysis* sp. It was found that 40 mg/L of chitosan is required to complete the flocculation process. On the other hand, 150 mg/L of chitosan was found to complete flocculation process of *Chaetoceros muellaris* [6]. Although chemical flocculation is often reported having less operating cost for algae harvesting method, however involving the chemicals on flocculation process can significantly hazardous to the environment [47]. The interruption of CO_2 supply to the algal system can cause algae to flocculate on its own, which is called autoflocculation. In most cases, this phenomenon was associated with elevated pH due to photosynthetic CO_2 consumption corresponding with precipitation of magnesium, calcium, phosphate, and carbonate salts with algal cells [48]. In the case of calcium phosphate used, excess calcium ions (positive charged) tend to reacts to algae cells (negative charged) and binds together to provide autoflocculation process [48].

3.2. Centrifugation

Centrifugation is definitely the preferred method for the harvesting on algal cells [42,49,50]. Centrifugation involves the application of centripetal acceleration to separate the algal growth medium into regions of greater and less densities. Once separated, the algae can be removed from the culture by simply draining the excess medium. Filters can also be implemented during centrifugation to separate the supernatant from the medium. Even though centrifugation is a plausible method to harvest microalgae, the shear forces experienced during spinning can disrupt cells, thus limiting the speed of centrifugation. Mohn [50] compared the appropriateness of different makes and brands of centrifuges for the dewatering of microalgae. Key parameters involved with the study included the concentration factor produced, energy consumption, relative cost, operation mode, concentration method and reliabilty. An early works on separation of algae by centrifugation has been documented in the literatures, though not extensively. Sim et al. [51] compared different techniques in harvesting microalgae and found that the centrifugation is as most efficient method for biomass recovery as compared to other techniques such as dissolved air flotation and drum filtration. Later, Heasman et al. [6] reported 88–100% cell viability and around 95–100% harvesting efficiency by centrifugation at 13,000 $\times$ *g*. Nevertheless, laboratory centrifugation was reported more suitable when the concentrations of the suspended sediment above 30 mg/L [52]. Although the centrifugal methods are highly reliable in production of metabolites, it does have some limitations. High gravitational and shear forces during the centrifugation process will damage cell structure. Additionally, it is not cost effective due to high power consumption especially when considering large volumes.

3.3. Filtration

Filtration is the method of harvesting that has proved to be the most competitive compared to other harvesting options. There are many different forms of filtration, such as dead end filtration, microfiltration, ultra filtration, pressure filtration, vacuum filtration and tangential flow filtration (TFF). Generally, filtration involves running the broth with algae through filters on which the algae will accumulate and allow the medium to pass through the filter. The broth will be continually run through the microfilters until the filter contains a thick algae paste [53].

It has been recognised that the use of filter presses under pressure or a vacuum are adequate methods to concentrate strains of microalgae that are considered to be large such as *Spirulina plantensis*. The recovery of small dimensioned algae strains such as *Dunaliella* and *Chlorella* which have cell dimensions similar to that of bacteria cannot be recovered by the pressure or vacuum filtration methods. Mohn [50] has compared different makes and brands of pressure and vaccum filtration units for their approriateness for the dewatering of microalgae. Mohn's [50] study warned that the pressure belt filter and vacuum filter thickener were not appropriate for harvesting and their use was not recommended for use with *C. proboscideum*. Recent studies show that tangential flow filtration (TFF) and pressure filtration can be considered as energy efficient dewatering methods, as they consume adequate amounts of energy when considering the output and initial concentration of the feedstock [53]. Simple filtration methods such as dead end filtration are not adequate as dewatering methods on their own due to issues with back mixing. However, simple filters can be used in conjunction with centrifugation to create better separation. Both Mohn [50] and Danquah et al. [53] have presented data on the concentration factor and energy consumption of specific filtration units. Although filtration methods appear to be an attractive dewatering option, they are associated with extensive running costs and hidden preconcentration requirements.

4. Products from microalgae

4.1. Biodiesel

Biodiesel is made from vegetable oils and animal fats which consist of triglycerides. Latter comprise of three chains of fatty acids joined by a glycerol molecule. The biodiesel process replaces the glycerol with methanol, forming fatty acid methyl esters (FAME), which is called as biodiesel. Glycerol the by-product, can be separated from biodiesel by phase separation method. The process is called transesterification, which substitute methanol for glycerol in a chemical reaction, using an alkali/acid as catalyst. Biodiesel production to replace conventional fuel needs to have: (1) sufficient feedstock to produce fuel at commercial scale; (2) lower cost than conventional fossil fuel; (3) match standard specification of fuel quality. Based on that, microalgae are potential to be used as a raw material for biodiesel production as they possess high growth rate and provides lipids fraction for biodiesel production [54]. Microalgal lipids are mostly neutral lipids with lower degree of unsaturation. This makes microalgal lipids a potential replacement for fossil fuel. Table 3 shows oil content of some microalgae strains. Numerous methods for extraction of lipids from microalgae have been applied; but most common methods are expeller/oil press, liquid–liquid extraction (solvent extraction), supercritical fluid extraction (SFE) and ultrasound techniques. Table 4 shows the advantages and limitations of different extraction methods.

Oil presses or expellers are commonly used to extract oil from nuts and seeds [55]. Same equipment and process can be used to extract oil from microalgae. In order for this process to be effective, algae must first need to be dried. Press uses pressure to break cells and compress out oil. Although this method extracts almost 75% of oil and no special skills is required, this method was reported less effective due to comparatively longer extraction time [55].

Secondly, solvent extraction also proved to be successful in order to extract lipids from microalgae. In this approach, organic solvents, such as benzene, cyclo-hexane, hexane, acetone, chloroform are added to algae paste. Solvent destroy algal cell wall, and extract oil from aqueous medium because of their higher solubility in organic solvents than water. Solvent extract can then be subjected to distillation process to separate oil from solvent. Latter can be reclaimed for further use. Hexane is reported to be the most efficient solvent in extraction based on its highest extraction capability and low cost [22,62]. Besides, Fajardo et al. [63] improved lipid extraction with two step process by using ethanol

Table 3
The oil content of some microalgae species.

Microalgae	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77

Adapted from Chisti [27].

Table 4
The advantages and limitations of different extraction method.

Extraction methods	Advantages	Limitations	References
Oil press	Easy to use, no solvent involved	Large amount of sample required, slow process	[55]
Solvent extraction	Solvent used are relatively inexpensive; reproducible	Most organic solvents are highly flammable and/or toxic; solvent recovery is expensive and energy intensive; large volume of solvent needed	[56,57]
Supercritical fluid extraction	Non-toxicity (absence of organic solvent in residue or extracts), 'green solvent' used; non-flammable, and simple in operation	Often fails in quantitative extraction of polar analytes from solid matrices, insufficient interaction between supercritical CO_2 and the samples	[58,59]
Ultrasound	Reduced extraction time; reduced solvent consumption; greater penetration of solvent into cellular materials; improves the release of cell contents into the bulk medium	High power consumption; difficult to scale-up	[60,61]

(stage 1) and hexane (stage 2) in order to purify extracted lipid and resulting about 80% of lipid recovery yields. Butanol has also been shown effective in extracting lysophospholipids. However, butanol is difficult to evaporate and tend to extract more impurities because of its high polarity [64]. A study by Morrison and Conventry [65] showed that fatty acids were nearly always more extractable at 100 °C as compared to ambient temperature, particularly saturated acids (16:0, 18:0), but polyunsaturated acids (18:2, 18:3) gave slightly lower yields with hot propanol:-water (3:1, v/v), water saturated butanol, methanol and methanol:water (85:15, v/v). In addition, Pratoomyot et al. [66] found that fatty acid content in microalgae varied between different species when extracted using chloroform:methanol (2:1, v/v) as a solvent. However, this particular method creates harmful environment due to the consumption of destructive chemicals.

Furthermore, supercritical extraction makes use of high pressures and temperatures to rupture the cells. This particular method of extraction has proved to be extremely time efficient and is commonly employed [58]. Canela et al. [67] reported that temperature and pressure of SFE did not have any effect on yield of extracted compounds, but it has affected extraction rate. Andrich et al. [21] studied kinetics of SFE in extraction of *Nanochloropsis* sp. to produce bioactive lipid (polyunsaturated fatty acids, PUFA). PUFA profile was about the same when different SFE conditions applied (45 and 55 °C, 400–700 bar). However, SFE system and solvent extraction (hexane) were found to give a similar effect on lipid extraction. Further studies by Andrich et al. [68] with *Spirulina platensis* for extraction of PUFA found that SFE system gave higher yield and fatty acid composition compared to the solvent extraction.

Another promising apparatuses to be used in extraction of microalgae is via ultrasound. This method exposes algae to a high intensity ultrasonic wave, which creates tiny cavitation bubbles around cells. Collapse of bubbles emits shockwaves, shattering cell wall thus disrupting latter and releasing desired compounds into solution. Wiltshire et al. [69] extracted over 90% of fatty acids and pigments yields from *Scenedesmus obliquus*, using ultrasound. Neither breakdown nor alteration to products was observed during extraction process. Later, a research by Pernet and Tremblay [23] used ultrasound for complete extraction of lipids from *Chaetoceros gracilis*. Yield of lipid extracts was investigated according to storage time and treatment method. It was proved that ultrasonic increased extraction rate thus affected recovery of lipid extracts throughout study. Although extraction of oil from microalgae using ultrasound is already in extensive use at laboratory scale, but sufficient information on feasibility or cost for a commercial-scale operation is unavailable. This approach seems to have lot of potential, but more research is needed to be done.

4.2. Bioethanol

Bioethanol produced from biomass is commonly produced from: (1) biochemical process (fermentation) and (2) thermo-chemical process (gasification). Most of biomass feedstocks which generate bioethanol such as corn, and sugar cane have problems in common: high value for food applications and requires large quantities of land to be produced. The fact that land has many other usages, makes this problem particularly acute, and became a constraint to expand production of biofuel [70]. The current interests in producing ethanol are focusing on microalgae as a feedstock for fermentation process. Microalgae provide carbohydrates and proteins that can be used as carbon sources for fermentation. Table 5 shows the amount of carbohydrates and protein measured from different algal species. Bacteria, yeast or fungi are microorganisms used to ferment carbohydrates to produce ethanol under anaerobic conditions. Besides the ethanol as main products, carbon dioxide and water are also formed as by-products. In general, according to simplified reaction equation below, theoretical maximum yield is 0.51 kg ethanol and 0.49 kg CO_2 per kg of carbon sugar, glucose.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

Very less research work has been reported on the fermentation of algae for ethanol production. Moen [72] showed that brown seaweed produced higher bioethanol compared to other algae species. Another study by Hirayama et al. [73] proposed a self-fermentation of algae to obtain ethanol. This technique was simpler with shorter fermentation time compared to conventional fermentation. Ueda et al. [74] patented a detailed system for microalgae fermentation. In the first stage, microalgae were fermented in anaerobic and dark environment to produce ethanol. The ethanol produced from fermentation can be purified to be used as fuel and produced CO_2 was recycled to algae cultivation ponds as a nutrient to grow microalgae. The second stage involved the

Table 5
Amount of protein and carbohydrates from various species of microalgae on a dry matter basis (%).

Algae strains	Proteins	Carbohydrates
Scenedesmus obliquus	50–56	10–17
Scenedesmus quadricauda	47	–
Scenedesmus dimorphus	8–18	21–52
Chlamydomonas rheinhardii	48	17
Chlorella vulgaris	51–58	12–17
Chlorella pyrenoidosa	57	26
Spirogyra sp.	6–20	33–64
Dunaliella bioculata	49	4
Dunaliella salina	57	32
Euglena gracilis	39–61	14–18
Prymnesium parvum	28–45	25–33
Tetraselmis maculate	52	15
Porphyridium cruentum	28–39	40–57
Spirulina platensis	46–63	8–14
Spirulina maxima	60–71	13–16
Synechoccus sp.	63	15
Anabaena cylindrical	43–56	25–30

Adapted from Becker [71].

utilization of remaining algae biomass after fermentation and used in anaerobic digestion process. This process produced methane which can further converted to produce electricity [74]. Later, Bush and Hall [75] patented fermentation process by adding yeast, *Saccharomyces saravesei* to algae fermentation broth for ethanol production. A study by Hon-Nami [76] indicated that *Chlamydomonas perigranulata* was fermented to produce ethanol, butannediol, acetic acid and CO_2. They found that hydrogen recovery from that fermentation was about 139% and carbon recovery at around 105%. Even though limited reports on algae fermentation were observed, a number of advantages were observed in order to produce bioethanol from algae. Fermentation process requires less consumption of energy and simplified process compared to biodiesel production system. Besides, CO_2 produced as by-product from fermentation process can be recycled as carbon sources to microalgae in cultivation process thus reduce the greenhouse gases emissions. However, the production of bioethanol from microalgae is still under investigation and this technology has not yet been commercialized.

4.3. Biomethane

The application of methane fermentation technology to algae has received considerable attention because it produces valuable by-products such as biogas. Biogas mainly consists of a mixture of methane (55–75%) and carbon dioxide (25–45%) produced during anaerobic digestion by anaerobic microorganisms. Methane from anaerobic digestion can be used as fuel gas and also be converted to generate electricity [77]. Residual biomass from anaerobic digestion also can further reprocessing to make fertilizers. In addition to being renewable and sustainable, this would encourage sustainable agricultural practices in providing greater efficiencies and reduce algae production costs. Microalgae contain almost no lignin and lower cellulose; therefore demonstrate good process stability and high conversion efficiencies for anaerobic digestion [24]. Table 6 shows methane yield produced from different algae species as feedstock. The biogas production from this anaerobic digestion process is primarily affected by its organic loadings, temperatures, pH and retention time in reactors. Basically, long solid retention time and high organic loading rate give significant results in high methane yield [78]. In addition, anaerobic digestion can operate in either mesophilic (35 °C) or thermophilic (55 °C) conditions. Otsuka and Yoshino [83] used constant temperature, mesophilic for anaerobic digestion of *Ulva* sp. and found 180 mL/g (volatile solid based) of methane yield. On the other hand, Golueke et al. [84] reported that mesophilic condition promoted slower breakdown of organic compounds in anaerobic digestion process. However, it was reported that production cost of methane from microalgae was higher compared to other biomass, grass and wood [82]. The integrated processes that combine algae cultivation and wastewater treatment system for methane production can be most suitable approach to reduce production cost and make it more profitable. Oswald and Gotaas [85] first reported use of wastewater ponds to cultivate algae and harvested algal sludge was anaerobically digested to produce biogas. Since then, a few works have been done to study in details about this system [86]. It can be concluded that system could avoid eutrophication process and improve pond waste nutrient treatment [87]. Although microalgae offer a good potential for biogas production, commercial productions have still not been implemented.

Table 6
Methane yield from different algae strains.

Biomass	Methane yield ($m^3 kg^{-1}$)	Reference
Laminaria sp.	0.26–0.28	[78]
Gracilaria sp.	0.28–0.4	[79]
Macrocystis	0.39–0.41	[78]
L. Digitata	0.5	[80]
Ulva sp.	0.20	[81]

4.4. Fine organic chemicals

In addition to ethanol production, certain other biochemicals in algae are ideal as feedstock for fermentation by microbes to produce fine chemicals. An important example is biobutanol and acetone. Biobutanol is a valuable organic solvent and has a potential to be used as a renewable transport fuel. The production process of biobutanol through acetone butanol (AB) fermentation using bacteria, normally *Clostridium* sp. is commonly alike to the process of bioethanol. Biobutanol was reported to have higher energy contents compared to ethanol [88]. In application as alternative fuel, biobutanol can readily be used in vehicles without any engine modifications since it can be blended in higher concentrations with gasoline compared to other biofuels. Besides, it can easily be added to conventional gasoline due to lower vapour pressure (www.bp.com). Also, acetone is common by-product during AB fermentation. It can be used as a solvent for many purposes; extractions, cleaning agents and for laboratory uses. Microalgae provide carbohydrate fraction thus has potential to be used as a feedstock in AB fermentation process and sharing a similar role with conventional AB fermentation feedstock such as grains and molasses. Although biobutanol and acetone have high prospects, little effort on the fermentation of microalgae for biobutanol and acetone was observed. Nakas et al. [89] fermented five species of *Dunaliella* sp. with *Clostridium pasteurianum* to generate different kinds of solvents (n-butanol, propanediol and ethanol). They found that use of microalgae as a substrate achieved about 14–16 g/L of mixed solvents. Meanwhile, the yield of fermented products using microalgae species was improved compared to classical *C. pasteurianum* fermentation [89]. Nevertheless, further research needs to be done in order to understand the details of the process in producing those chemicals from microalgae.

4.5. Food and food supplements

4.5.1. Omega 3 oil

Microalgae naturally contain omega-3 fatty acid which can be purified to provide a high value food supplement. The functional sources of omega-3 in microalgae are normally eicosapentanoic acid (EPA) and decosahexaenoic acid (DHA). Omega-3 fatty acids are widely obtained from fish oil, but in recent years, problems arise with unpleasant taste and poor oxidative stability of fish oil, making it less favourable [90]. Also, inadequate of fish oil supplies contribute to limit its use. In comparison to fish, microalgae are self-producing omega-3 thus, making the process simple and economical [91]. EPA has been used in clinical purposes, such as for treatment of heart and inflammatory diseases; asthma, arthritis, migraine headache and psoriasis [92]. A number of literatures have been reported the mass culture of microalgae to produce EPA. Hu et al. [93] cultivated marine microalga, *Pavlova viridis* using 60L outdoor photobioreactor and made comparison with indoor cultivation. They observed that outdoor photobioreactor system gave a lower total fatty acid but higher in EPA compounds compared to indoor system. Hence, they concluded that outdoor system can be applied for EPA production method [93]. Also, Cheng-Wu et al. [94] used outdoor photobioreactor to cultivate *Nannochloropsis* sp. and produced EPA. The seasonal variations affected EPA yields; up to 35% higher yield in summer as compared to that in winter. Besides, Chini Zittelli et al. [95] also cultured *Nannochloropsis* sp. and found that photobioreactors gave more

advantages in controlling contaminants compared to open pond system. Temperature and irradiance of photobioreactors was indicated not much affected the EPA yields [95]. Nevertheless, cost effective system for culturing microalgae is needed to be developed in order to meet demands of EPA. Similarly, DHA is used for health benefits: helps to fight cancer, AIDS, heart disease, lower cholesterol, boost immune system, and detoxify body. Amount of DHA produced have significantly been affected by types of microalgae. It was studied that marine microalgae having significantly more DHA contents compared to fresh water microalgae; mainly consists saturated or monounsaturated fatty acids [96]. *Schizochytrium mangrove*, marine microalgae was reported to have main component of DHA in a range of 33–39% of total fatty acid [97]. Vazhappilly and Chen [98] confirmed that *Crypthecodinium cohnii* had DHA content up to 19.9% of total fatty acid than other microalgae species studied; *Amphidium caryerea* (17.0%) and *Thrautocytrium aureum* (16.1%). Also, *Isochrysis galbana*, was found to have significant amount of DHA with the specific productivity around 0.16 g/L d [96]. Besides, a study by Carvanho and Malcata [99] indicated that amount of CO_2, light intensity, operation mode (batch and continuous) significantly affect productivity of DHA. Almost 1.29 mg/L d of DHA was obtained under optimized conditions.

4.5.2. Chlorophyll

Microalgae produce of wide variety of biomaterials, one of them is chlorophyll. Most algae cultured under optimum condition were reported contained about 4% dry weight of chlorophyll from overall cell weight (reported by Solar Energy Research, 1984). Cyanobacteria (known as blue green algae) typically contain chlorophyll-*a* while species of green algae mostly have chlorophyll-*b* [100,101]. Among species of microalgae, *Chlorella* was reported to have high amount of chlorophyll [102]. Chlorophyll provides a chelating agent activity which can be used in ointment, treatment for pharmaceutical benefits especially liver recovery and ulcer treatment. Besides that, it repairs cells, increases haemoglobin in blood and faster the cell growth [103]. Chlorophyll has also been investigated as source of pigments in cosmetics. The brown and red algae are mostly used in the cosmetics industries [104]. Furthermore, in food industry, chlorophyll is used as natural pigment ingredient in processed foods [105]. Because of its strong green pigment and consumers demand for natural foods, chlorophyll is gaining importance as food additive. This in turn is encouraging food processors to switch from artificial pigments to chlorophyll-based natural colouring. However, a down stream process needs to be developed to purify chlorophyll *a* and *b* from algae.

4.5.3. Livestock feed

Another useful commodity from algae is livestock feed. A large number of algae have been tested for their biochemical compositions to be used as livestock feed supplement or as primary livestock feed. Edible seaweeds have reported to be used as food due to lower calorie, high concentration of minerals, vitamins and proteins and a low fat content [106]. *Spirulina* a well-known blue green alga, is still used in food supplements due to its excellent nutrient compounds and digestibility [107]. Besides higher content of protein (60–70 wt%), *Spirulina* also contains a rich source of vitamins, especially vitamin B_{12} and provitamin A (β-carotene) and minerals [108]. Compared to other microorganisms, *Spirulina* can be cultivated in high saline water and alkaline conditions which give an advantage to function as a feedstock for livestock feed. In addition, Red algae, mainly *Porphyra* and brown algae, particularly *Laminaria*, *Undaria*, and *Hizikia fusiforme* were directly consumed in human food [109]. *Chlorella* has been suggested as such potential food, principally because it consists of complete nutrients for food [2]. Moreover, microalgae also play a key role in high grade animal nutrition food, from aquaculture to farm animals. Comprehensive nutritional and toxicological evaluations have demonstrated suitability of algae biomass as a valuable feed supplement or substitute for conventional animal feed sources [106]. Microalgae species *Hypnea cervicornis* and *Cryptonemia crenulata* particularly rich in protein were tested in shrimp diets [110]. Amount of algae in fish meal resulted in significant increase in shrimp growth rates. In addition, better growth weight and protein efficiencies ratio of *Tilapia* fish was observed when supplied with algae as nutritional food source in feed [111]. Also, *Phorphidium valderianum*, marine cyanobacteria were successfully used as feed for aquaculture based on their nutritional and non-toxic performance [108]. In addition to its importance in aquaculture, algae were reported to contain up to 5–10% of proteins which can directly be used to replace conventional protein sources in poultry feed [2]. Ginzberg et al. [112] studied role of algae, *Porphyridium* sp. as feed supplement on metabolism of chicken. It was found that cholesterol of egg yolk was reduced about 10% and colour of egg yolk became darker, indicating higher carotenoid was produced. Belay et al. [113] reviewed potential of *Arthrospira* (*Spirulina*) in animal feed. Although *Arthrospira* is widely used as food additive and can replace 50% of protein diets in existing feeds, it was concluded that protein sources from soya and fish meal were preferable compared to *Arthrospira* because there were more cost effective [106]. Besides that, a study on the addition of *Laminaria digitata* suggested that algae supplemented feed increased pig weight up to 10% on a daily basis [114].

5. Microalgae for environmental applications

Algae serve an advantage for effluent treatment via increasing performance of degradation, improving CO_2 balance and lowering energy demand for oxygen supply in aerobic treatment stages. The role of algae was both to assimilate plant nutrients and to support bacteria with oxygen. Bacteria, in turn, were involved in degradation of organic material in wastewater, same process utilized in activated sludge. Fig. 3 demonstrates process involved in a high rate algal pond. Cyanobacteria were reported to be effectively used for treatment of organic pollutants from paper industry wastewater [115]. Besides, wastewater containing organic compounds, phenol was efficiently removed with aid of algae culture [116]. Conventional phenol degradation, using bacteria, requires addition of external carbon sources for growth whilst microalgae use CO_2 as carbon sources (photoautrotophically) hence no addition of carbon sources to system is needed [117]. However, microalgae have limitations to be used in removal of organic compounds due to slower growth rate compared to bacteria [31]. Furthermore, microalgae are able to be used for removal of heavy metals in industrial wastewater. Brown alga,

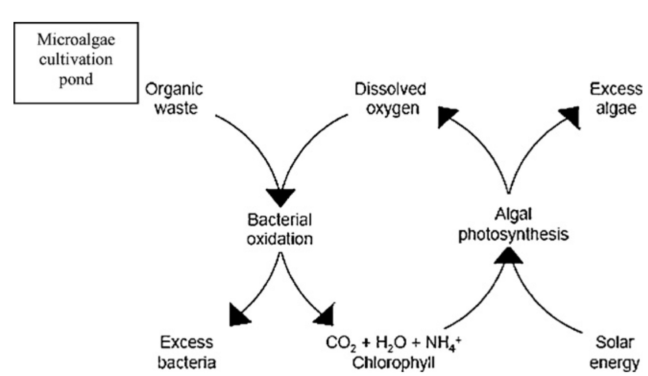


Fig. 3. The main processes involved in a high rate algal pond [85].

Ascophyllum nodosum has proven to be the most effective algal species to remove metals of cadmium, nickel, and zinc from monometallic solutions compared to green and red algae [29]. A similar study proved brown alga *Fucus vesiculosus* gave highest removal efficiency of chromium (III) at high initial metal concentrations [118]. In accordance with this, biochemical composition of cell wall (alginate and fucoidan) of brown algae was found have promising benefits for biosorption process. Another algal species, *S. obliquus* was examined for degrading cyanide from mining process wastewaters [119]. It was observed that cyanide was degraded up to 90% after introduction of algae into the system. *Spirogyra condensata* and *Rhizoclonium hieroglyphicum* also employed as biosorption substrates to remove chromium from tannery wastewater [119]. The pH and concentration of algae were concluded to have significant effect on removal of chromium thus indicating potential of algae for removal hazardous heavy metals in wastewater [119].

6. Conclusion

Based on this review, it is clear that microalgae have a potential to produce a wide range of products due to its high-quantity natural proteins, lipids, carbohydrates, vitamins, pigments and enzymes contents. The ultimate production system of microalgae includes cultivation and dewatering process could not be established so far due to insufficient commercial-scale operation. Although various commodities are produced from microalgae, extensive studies need to be done include upstream and downstream processes in order to compete with existing commodities. Besides, microalgae also have potential to be used in environmental applications such as removal of excess organic/inorganic nutrients and heavy metals. Further, algae is also currently gaining attention for being capable of significantly reducing greenhouse gases concentration, thus providing solution to global warming. Overall, the production of microalgae promotes global prospects and may provide sustainable economic development in future.

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